SYMPOSIUM ABSTRACTS

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THE CURIOUS TALE OF AMYLOID β-PEPTIDE: HOW SECRETION OF A TRANSMEMBRANE DOMAIN FRAGMENT LEADS TO ALZHEIMER'S DISEASE. D. J. Selkoe, Harvard Medical School and Brigham and Women's Hospital, Boston, MA 02115

Progressive cerebral deposition of the 40-42 residue amyloid β-protein (Aβ) is an early and invariant feature of Alzheimer's disease (AD). Aβ is released by proteolysis from the β-amyloid precursor protein (βAPP), a type I integral membrane glycoprotein that is ubiquitously expressed in mammalian cells. At least two genetic forms of AD --missense mutations in the βAPP gene on chromosome 21 and trisomy 21 (Down's syndrome) ---involve a causative role of βAPP metabolism and Aβ deposition in producing the disease. Other genetic factors linked to AD, including an as yet unidentified gene on chromosome 14 and the apolipoprotein E4 allele on chromosome 19, are also associated with increased brain Aβ deposition.

Besides its role in AD, Aβ is also a normal secretory product of cellular metabolism. The discovery that soluble Aβ peptides having heterogeneous N- and C-termini are normally secreted by cells has made possible the detailed mechanistic study of the regulation and pharmacological inhibition of Aβ production. Aβ is present at high pM to low nM levels in culture medium, CSF and plasma. Cellular generation of Aβ requires the membrane insertion and post-translational maturation of βAPP and occurs in acidic vesicles other than lysosomes, e.g., in the late Golgi or early endosomes. The unknown proteases that generate the N- and C-termini of Aβ are called β- and γ-secretases. Mutagenesis studies of βAPP expressed in cultured cells reveal a marked sequence specificity of β-secretase but not γ-secretase. Studies of βAPP trafficking in living neuronal and non-neuronal cells show that βAPP is endocytosed, rapidly recycled to the cell-surface, and ultimately trafficked in part to lysosomes. Manipulations that decrease the endocytosis of cell-surface βAPP decrease Aβ production, suggesting that a portion of βAPP molecules undergo β-secretase cleavage in early endosomes, followed by γ-secretase cleavage of the resultant 99-residue carboxyl terminal fragment of βAPP and rapid release of Aβ from the cell surface. The direct relevance of such in vitro studies of Aβ generation to the situation in vivo has been demonstrated by: a) detecting soluble Aβ peptides of identical lengths in human CSF and plasma; and b) finding quantitative and/or qualitative changes in Aβ secretion in primary donor cells expressing certain βAPP missense mutations linked to familial AD.

Cells that constitutively secrete Aβ are also useful for examining the critical issue of the aggregation of the $A\beta_{1-42}$ monomer into its neurotoxic polymeric form under physiological conditions. In this regard, we recently found that CHO cells expressing normal or mutant βAPP show aggregation of ≤10-20% of their secreted Aβ peptides into SDS-stable dimers, trimers and sometimes tetramers under normal culture conditions. The identity of these small oligomers has been confirmed by extensive immunochemical characterization and radiosequencing. Using this endogenous Aβ aggregating system, we have begun to examine variables that may influence aggregation as well as compounds which may retard it.

In conclusion, studies of Aβ production and aggregation in cell culture have provided information under biologically relevant conditions that complement analyses of these processes in animal models and in human CSF and point to novel therapeutic strategies for this complex disorder.

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